STUDIES ON BIORESPONSES OF LONICERAE FLOS

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I. Introduction

From ancient period, it is well known that Lonicerae Flos (Lonicera japonica) clears Heat and detoxifies Fire Poison, clears Damp heat in the Lower Burner. And it has been used for hot, painful sores and Swellings in varying stages of development, especially of the breast, throat or eyes. And its has also been used for Damp Heat dysentry-like disorder or painful urinary dysfunction (1). In mordern medicinal studies, this herb has been reported to show the antibacterial effects, and antiviral effects (2). It has been also been reported that Lonicerae Flos prevent blood aggregation and endothelium damage (3). And luteolin, the main component of this herb, has been reported to posses the antiperoxidative effects (4). From these facts, we supposed that this herb might affect the immune functions, and performed these studies.

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Ⅱ. Experimental Methods

1. EXPERIMENTAL ANIMALS

8 week-old, ICR, balb/c and C57BL/6 mice were used. These mice were maintained under controlled environmental conditions (air filtered room, 21-24°C, lighting; 7:00-19:00 H) and allowed free acess to food and water.

2. MATERIALS

Herb was disintegrated and extracted in boiling water for 6 hours. After centrifugation, supernatant was concentrated and dried to be powder. Sample was dissolved in saline to make 1mg/ml and diluted with culture media to the appopriateconcentration before use. Different amounts of sample were addedto the medium at the beggining of the culture, otherwise mentioned.

3. ARTHUS REACTION AND DELAYED HYPERSENSITIVITY

Male ICR mice were sensitized by subcutaneously injecting 100g of bovine serum albumin emulsified in complete Freund's adjuvant. Seven days later delayed hypersensitivity and arthus reaction were elicited by challenging mice in the footpad with heat aggregated BSA in saline, as previously described (7, 8, 9). At 3 and 24 hours post-challenge, footpad swelling thicknessw was measured with micrometer and the extent of swelling was calculated by substracting the thickness of the negative foot pad from that of antigen injected footpad.

4. IN VIVO PHAGOCYTOSIS.

Intravascular phagocytosis by reticuloendothelial system, particulary kupper cell and splenic macrophage, was evaluated in ICR mice. Colloidal carbon (80 mg/ml) suspendedin 1 % gelatin-saline was injected intravenously at adose of 16 mg per 100g of body weight, as previously described (10, 11). Blood was

collected from retroorbital plexus at 5 mininterval after carbon administeration. Blood was lysed in 0.1 % sodium carbonate and carbon absorbances were determined using a UV spectrophotometer. Phagocytosis was expressed as the phagocytic index and corrected phagocytic index.

5. PLAQUE FORMING CELL ASSAY

Splenic IgM antibody forming cells to thymic dependent antigen, sheep RBC were quantified by the modified liquid monolayer slide method. 4 days following a single intraperitoneal injection with 2 % suspension of sheep RBC in male Balb/c mice (12, 13). Spleen single cell suspension was prepared in BSS(PH 7.2) using a stainless sieve and adjusted to a cell concentration of 2 x 10^7/ml. Reaction mixture was composed of spleen cell suspension, 12.5 % sheep RBC, 1/5 diluted complement and BSS. Reaction mixture was pipetted into each chamber in triplicate per sample.

6. PREPARATION OF CELL SUSPENSION

Spleen were removed and placed in RPMI 1640 media(Sigma) supplemented with 100 U/ml penicillin and 100 ug/ml streptomycin, 0.2 mM sodium pyruvate, 2 mM glutamine, 10 mM HEPES, 2 g/L sodium bicarbonate, 1 mM nonessential amino acids, 50 uM 2-metrcaptoethanol (this media refer to as modified RPMI 1640 media). Spleen cell suspension, obtained by dissagregation was washed by centrifugation, and RBC was lysed by hypotonic shock.

7. LYMPHOPROLIFERATION

Spleen cells were cultured in modified RPMI 1640 media supplemented with heat inactivated FBS ,and stimulated with 5 ug/ml Con A (Sigma Type III). Cultures were incubated for 30 hours and were pulsed with 0.5 uCi ^{3}H-thymidine. Cells were collected and radioactivities were determined by scintillation counter.

8. TCGF PRODUCTION

Spleen cells were cultured in modified RPMI 1640 media in the presence of 5 ug/ml ConA. After 24 hours of incubation at 37°C in a humidified CO_2 incubator, supernatants were harvested and stored at 20°C until used. Supernatants were assayed for TCGF by their ability to induce proliferation of ConA-activated T cell blasts as described by Coutinho, Larson, Gronvik and Anderson(14).

9. STATISTICAL ANALYSIS

The significance of the differences was evaluated by student's T-test.

III. RESULTS AND DISCUSSION

In our study, we examine the effect of Lonicerae Radix extract (LFE) on immune function and evaluate its possibilities as potentiator or suppressor of immune response induced by exogeneous influences. The effects on the capacity of B cell and macrophage to cooperate in the production of antibody to thymic dependent antigen, sheep RBC (SRBC) as humoral immunity, were evaluate in nor,mal Balb/c mice.

Table I. Effects of LFE on the IgM plaque forming cells in normal male Balb/c mice*.

GROUP	IgM PFCs/10^6 spleen cells	IgM PFCCs/spleen
Control	687 + 95	61,400 + 8,700
LFE (10mg/kg)	710 + 89	67,500 + 7,300
LFE (25mg/kg)	669 + 114	60,900 + 10,600

* Mice were immunized with 0.2 ml of 2 % SRBC ($4x10^8$ cells/ml) intraperitoneally. Four days after the immunization, assay was amde. The values represent the arithmetic mean + standard error from 5 mice.

Table II. Effects of LFE on the in vivo phagocytosis carbon as macrophage function in male ICR mice.

GROUP	Phagocytic index (P.I)	corrected P.I.*
Control	3.05 + 0.32	5.94 + 0.27
LFE (10mg/kg)	2.72 + 0.28	5.66 + 0.30
LFE (25mg/kg)	2.68 + 0.34	5.57 + 0.36

* Phagocytic index and corrected phagocytic index was calculated according to Stuart's methods. The data represent the arithmetic mean + standard error.

Table III. Effects of SRE on the arthus and delayed hypersensitivity(DH) reaction in male ICR mice*.

GROUP	Arthus(footpad)	DH(swelling thickness, 10^{-1}mm)
Control	7.68 + 0.73	8.32 + 0.64
LFE (10mg/kg)	8.57 + 0.76	6.04 + 0.78
LFE (25mg/kg)	7.85 + 0.60	6.52 + 0.91

* Groups of ICR mice were sensitized to 100 ug BSA in CFA subcutaneously. Seven days following sensitization, the mice were challenged with saline or 30 ul of 2 % HA-BSA in the footpad. At 3 and 24 hours after challenge the increase in thickness of the footpad was determined.

Table IV. Effects of LFE on ConA induced lymphocyte proliferation in normal C57BL/6 mice.

GROUP	H-thymidine uptake(cpm x 10^{-3})
Control	68.75 + 7.93
LFE (0.1mg/ml)	62.31 + 4.89
LFE (1.0mg/ml)	52.58 + 6.07

Table V. Effects of LFE on TCGF secretion of spenocytes of C57BL/6 mice.

GROUP	TCGF (U/ml)*
Control	116.8 + 13.4
LFE (25mg/kg)	104.3 + 17.2

* TCGF were assayed from supernatant of culture medium incubated for 24 with conA, 5ug/ml.

Treatment of LFE did not show any significant effect on PFCs in high and low dose of LFE (Table I). And , the affects of LFE on arthus reaction in nolrmal male ICR mice and thymus dependent antigen, SRBC, in normal balb/c mice were examined for the purpose of evaluation of the humoral immunity. AMH was induced by challenging with 2 % HA-BSA. Mice presenttized with Bovine serum albumine in Freund's complete adjuvant (FCA) have the high contents of circulating antibodies. Aggregates of precipitating IgG and IgA class stimulate the release of lysosomal enzymes in neutrophil. Challenged antigen-antibody complexe in blood vessels fix and activate complement. Neutrophil chemotaxis by Csa, C, and phagocytosis by those complexes are followed by the secretion of injurious inflammmatory mediators from neutrophil. Arthus reaction was not altered by treatment of

LFE (Table III). From these results (Table I, III), we suppose that LFE may not involved the synthesis or secretion of precipitating IgG antibody, complement fixation, and neutrophil chemotaxis. Delayed hypersensitivity (DH) reaction involves the sequential recognition of challenged antigen by specific presensitized T cells, immobilization of T cells at the site of challenge of followedd by production and release of lymphokines which mediate accumulation of macrophage, vasodilation and ultimate destruction of challenged antigen. Measurement of the thickness of swelling foot pad in mice has been known to be quantitative, sesitive, and simple method to perform accurately immunomodulation induced by chemicals. LFE showed inhibitory effect on delayed hypersensitivity reaction (table III).

In order to elucidate the precise mechanism of LFE on lymphocyte proliferation and release reaction, *in vitro* test were performed. Normal splenocytes were incubated at 30°C for 30 hours. Mitogen induced lymphocyte proliferation was performed with 5 ug/ml conA and LFE. And results were expressed as H-thymidine uptake into spenocytes. LFE significantly suppressed the conA-induced proliferation of splenocytes (Table IV). The precise action mechanism of LFE was not yet understood. But, there are many report on this effect of plant material(15). It is well known that IL-2 receptor or TCGF expression is necessary in cell proliferation (16, 17). As shown in Table V, LFE slighly, not significantly, decreased TCGH levels in the supernatant from the conA-stimulated splenocytes. These result was consistent with Table III, regarding the result that LFE decreased the delayed hypersensitivity. Therefore, LFE might decrease the synthesis and/or release of lymphokines from immune cells. The involvement of macrophages in immune responses have been well known for many years (18, 19). The effects of LFE on nonspecific phagocytic function of macrophages and kupper cells were presented in Table II. LFE slightly decreased phagocytic index and corrected phagocytic index, though not significant in statistics. In conclusion, lymphocyte function might be inhibited by LFE. But, the precise nature of this herb on immune cells should be further investigate.

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